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METHODOLOGICAL ARTICLE

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# Optimization of the sampling method to monitor *Drosophila suzukii* infestation in vineyards

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## Abstract

*Drosophila suzukii* is a highly polyphagous pest species that can also attack grape. To prevent economic damage in vineyards, it is important to detect *D. suzukii* infestation early in order to protect grapes efficiently. Here, we firstly examined if peripheral zones adjacent to natural habitats and central zones within a vineyard differ in infestation levels, and, secondly, where within a grape cluster most eggs are laid. Among the eight infested vineyards studied, seven had higher *D. suzukii* infestation levels in peripheral zones adjacent to hedgerows, forests or gardens. Females laid 2.0-fold more eggs on berries in the interior of grape clusters than on berries on the exterior of the cluster. Based on the finding that berries in the interior of grape clusters are more vulnerable, we developed a novel sampling method to estimate *D. suzukii* infestation level in vineyards more effectively. The so-called 'whole cluster method' consists of a representative but random collection of five grape clusters per plot and a visual inspection of five berries each from the inner and outer part of a cluster totalling 50 checked berries per plot. We then compared our newly developed method to two established sampling methods to estimate *D. suzukii* infestations (i.e. 'single berry method' and 'cluster fragment method'), which rarely collect and inspect berries from the interior of clusters. In 87 comparisons conducted in 35 different plots, the 'whole cluster method' was the most sensitive sampling method as the calculated mean infestation rate was highest and it identified eggs earlier and in more samples than the other two methods. We therefore believe that the 'whole cluster method' is currently the most effective method to assess *D. suzukii* infestations in commercial vineyards.

KEYWORDS
IPM, pest detection, spotted wing drosophila, survey, viticulture, *Vitis vinifera*

## 1 | INTRODUCTION

The spotted-wing drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), is an invasive Asian species that has become a major agricultural pest ever since it has spread around the world (Asplen et al., 2015; Cini et al., 2012; Walsh et al., 2011). Contrary to native vinegar flies, *D. suzukii* females have a serrated, sclerotized ovipositor that allows them to lay eggs in ripening, intact fruits of a wide range of wild and cultivated plant species (Kenis et al., 2016; Lee et al., 2011,2015; Poyet et al., 2015). Although past experiences show that grapevine is a secondary host plant species, *D. suzukii* females can lay eggs on many grape cultivars (Entling et al., 2019; Ioriatti et al., 2015; Kehrli et al., 2017; Mazzetto et al., 2020; Pelton et al., 2017; Shrader et al., 2018; Van Timmeren & Isaacs, 2014; Weissinger et al., 2019). In years with suitable weather conditions, these eggs might trigger the development of sour rot, which thereafter might impact the flavour and quality of processed wines (Barata et al., 2011; Entling & Hoffmann, 2020; Ioriatti et al., 2018; Rombaut et al., 2017). To prevent economic damage, it is therefore important to detect *D. suzukii* infestation on grapes early in order to protect vineyards (Ebbenga et al., 2019; Hall et al., 2018; Knapp et al., 2019; Linder et al., 2020). Since the presence of *D. suzukii* females in the vineyard and oviposition in grapes is not directly linked, trap captures provide little valuable information on pest pressure (Kirkpatrick et al., 2017; Mazzetto et al., 2020; Pelton et al., 2017; Weissinger et al., 2019). The EPPO guidelines advise to dissect and record the percentage of infested fruits by larvae and pupae for the evaluation of efficacy trials, and this can also be done by immersing fruits in a 10% NaCl solution (EPPO, 2013). The so-called salt bath method or fruit-dunk test has also been occasionally used to assess grape infestation (Pelton et al., 2017), but this sampling method is designed to detect larvae at the earliest several days after the actual oviposition. In addition, the detected larvae can usually not be distinguished with certainty from other drosophila species. To cope with this uncertainty, collected fruits can be stored in closed containers and emerging flies can thereafter be collected and identified (Van Timmeren & Isaacs, 2014). This method provides also a time-delayed assessment of grape infestation in vineyards and underestimates the actual infestation since only around 10% of laid *D. suzukii* eggs in grapes actually develop into adults (Linder et al., 2014). Yet, eggs found in intact grapes can without any time delay undoubtedly be assigned to *D. suzukii* since European vinegar flies are not able to pierce the berry skin and lay eggs. The monitoring of egg depositions in grape berries provides, therefore, the most reliable information on the actual infestation pressure by *D. suzukii* and the potential need to take additional control measures (Weissinger, Schrieber, et al., 2019). There exist currently two established sampling methods to assess egg infestation in grapes in Europe. First, the 'single berry method' that was traditionally used in Switzerland and Italy (Kehrli, Cahenzli, et al., 2017; Mazzetto et al., 2020) and that is based on a random collection and inspection of individual healthy grape berries in a vineyard. Second, the 'cluster fragment method', which is widely used in Germany and South Tyrol (Weissinger, Schrieber, et al., 2019) and that collects randomly grape cluster parts within a vineyard and from this pool of collected berries, single healthy grape berries are thereafter randomly selected and inspected for *D. suzukii* infestation.

In this study, we aimed to optimize the current sampling methods to assess egg infestation. First, we studied the place of oviposition by determining if peripheral zones adjacent to natural habitats differ in infestation levels to central zones within a vineyard and by identifying where eggs are actually laid in grape clusters. Second, we developed the novel 'whole cluster method' and compared its sensitivity to the two traditional sampling techniques 'single berry method' and 'cluster fragment method' in order to estimate more appropriately *D. suzukii* infestation in commercial vineyards.

## 2 | MATERIALS AND METHODS

### 2.1 | Site of oviposition

In 2017, we followed the temporal course of *D. suzukii* infestation in 10 commercial vineyards in the cantons Ticino and Vaud (for further details see 'Raw data Site of oviposition.xlsx' on https://doi.org/10.5281/zenodo.5181421). Except for the application of mating disruption against the two grapevine moths *Lobesia botrana* (Denis and Schiffermüller) and *Eupoecilia ambiguella* (Hübner) (Lepidoptera: Tortricidae), insecticides were usually not applied within the vineyards and grapevines were trained with the Guyot pruning system. To better understand the spatial distribution of eggs within vineyards as well as on grape clusters, we visited each of the ten vineyards between one and five times resulting in overall 34 different sampling occasions.

It is widely accepted that *D. suzukii* adults can spill over and infest adjacent crops from common landscape elements such as gardens, orchards, hedgerows and forests that contain wild host plants of *D. suzukii* and harbour important fly populations (Cahenzli et al., 2018; Haro-Barchin et al., 2018; Hennig & Mazzi, 2018; Kenis et al., 2016; Klick et al., 2016; Leach et al., 2019; Mazzetto et al., 2020; Pelton et al., 2016; Santoiemma et al., 2019; Tonina et al., 2018; Weissinger, Schrieber, et al., 2019). Taking into account such landscape elements and alternative host plants surrounding each vineyard, we defined a priori for each plot a zone of higher *D. suzukii* infestation risk and a zone of lower risk. Areas at the border of vineyard plots adjacent to hedgerows, forests or gardens harbouring alternative host plants of *D. suzukii* were thus considered as zones of higher risk, whereas areas situated in the centre of plots further away from these favourable landscape elements were considered as zones of lower risk. For a plot, we collected at each sampling occasion randomly five healthy grape clusters in its specified zone of higher risk as well as five healthy grape clusters in its defined zone of lower risk totalling 10 clusters per plot. Thereafter, we visually inspected grapes under the stereomicroscope at a magnification of at least 8 times for the presence of *D. suzukii* eggs. From each grape cluster, 5 healthy and intact berries from the interior of the cluster and 5 healthy and intact berries from the exterior were inspected. For each berry, we noted if *D. suzukii* eggs were present or absent.

In two of the 10 vineyard plots, no oviposition was observed over the different sampling occasions. Due to the lack of *D. suzukii* infestation in these two plots, we excluded them from further analyses in order to get a more accurate understanding on the actual spatial distribution of laid eggs within vineyards and on grape clusters. Since the other eight vineyard plots were sampled between 3 and 5 times, data for a single plot were pooled and the average infestation rate was calculated in both 'zone of risk' for the two 'position of berries on the cluster' totalling in four independent mean values per plot. The average 'percentage of infested berries' for a specific position on the grape cluster (interior versus exterior) for a zone of risk in a vineyard plot was used in the analysis as the dependent variable. 'Plot', the 'position of berries on the cluster' and the 'zone of risk' were treated as nominal independent factors. Data were analysed using a three-way ANOVA (R version 4.0.2), and the fulfilment of the model assumptions was checked by visually inspecting the distribution of the residuals.

## 2.2 | Comparison of egg sampling methods

In 2018 and 2019, we compared the two traditional sampling methods to estimate *D. suzukii* infestation (i.e. 'single berry method' and 'cluster fragment method') with the newly developed 'whole cluster method' in order to assess their sensitivity. We compared the three methods with each other for a total of 35 different plots and 87 sampling occasions (for further details see 'Raw data Method comparison.xlsx' on https://doi.org/10.5281/zenodo.5181421). With the exception of mating disruption against grapevine moths, no insecticides were usually applied within the 35 vineyards and grapevines were predominantly trained in the Guyot pruning system.

The 'single berry method' is usually based on a random collection of 50 healthy, individual grape berries in a vineyard (Kehrli, Cahenzli, et al., 2017; Mazzetto et al., 2020). Thereafter, these 50 berries are visually inspected under the stereomicroscope for the presence of *D. suzukii* eggs in order to calculate the percentage of infested berries. In the 'cluster fragment method', around 25 grape cluster parts (each consisting of 5 to 20 berries) are randomly collected per plot and pooled (Weissinger, Schrieber, et al., 2019). Fifty healthy berries are thereafter randomly selected from this pool for visual inspection of infestation using a stereomicroscope. The novel 'whole cluster method' consists of a random sampling of only 5 grape clusters per plot. Subsequently, 5 random healthy berries from both the interior and exterior of each cluster are visually inspected under the stereomicroscope to evaluate the percentage of infested fruits. In addition to infestation rates, we also estimated once for all three evaluation methods the weight of collected samples as well as the total time to collect grapes in a vineyard and to inspect berries in the laboratory.

Data on the number of samples 'with egg detection', 'with first egg detection for a plot', 'without egg detection by other two methods' and 'with highest infestation' for each method were compared with each other using $\chi^2$-tests followed by Bonferroni pairwise comparisons (R package RVAideMemoire v 0.9–73). The actual infestation rate was only calculated for vineyard plots with observed oviposition, and it was also averaged for a vineyard plot since the number of sampling occasions ranged between 1 and 5 times for a single plot. The average 'percentage of infested berries' for a method in a vineyard plot served as the dependent variable and was analysed by

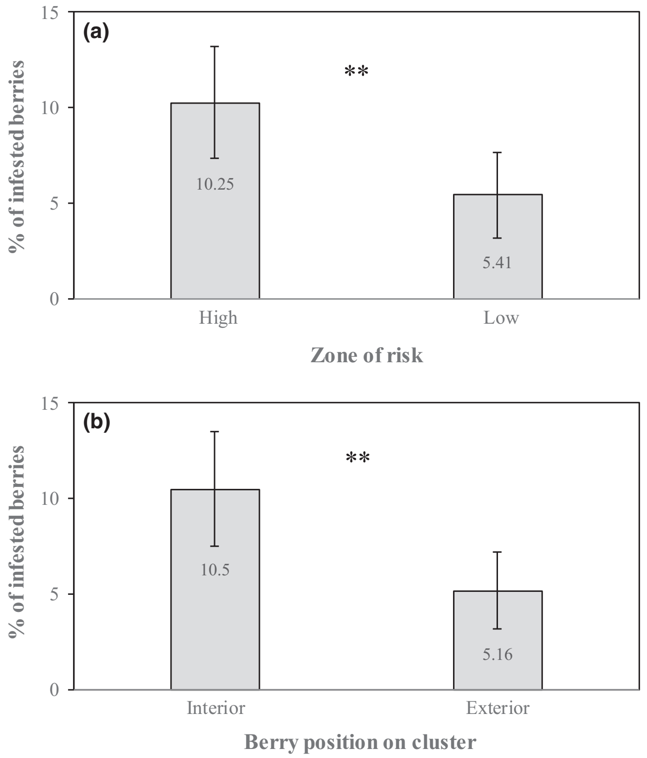


FIGURE 1 Percentage of berries infested by *D. suzukii* with respect to (a) the zone of infestation risk within the vineyard and (b) the position of berries within the cluster in 2017. Indicated are mean values ± SE ($n = 8$ plots), (3-way ANOVA, $^{**}p < 0.01$)

a two-way ANOVA using 'plot' and 'method' as nominal independent factors (R version 4.0.2). Thereafter, differences between the three methods were analysed using Bonferroni pairwise comparisons. The 'percentage of infested berries' was arcsine-transformed to fit normal distribution, and the fulfilment of the model assumptions was checked by visually inspecting the distribution of the residuals.

# 3 | RESULTS AND DISCUSSION

## 3.1 | Site of oviposition

The 'percentage of infested berries' was significantly affected by the 'plot' ($F_{7,21} = 21.9$, $p < 0.001$), the 'zone of risk' ($F_{1,21} = 10.9$, $p = 0.003$) and the "position of berries on the cluster" ($F_{1,21} = 13.3$, $p = 0.002$), whereas the interaction of the latter two factors was not significant ($F_{1,21} = 0.8$, $p = 0.38$). Zones of higher *D. suzukii* infestation risk could accurately be predicted in seven of the eight infested plots with 1.9 times more egg depositions in the zone at higher risk compared with the zone of lower risk (Figure 1a). Similarly, females laid 2.0 times as many eggs on berries from the interior of grape clusters than on outer berries (Figure 1b).

Vineyards are often in direct proximity of hedgerows, forests, orchards and gardens. These landscape elements usually contain wild host plants of *D. suzukii* that harbour important fly populations, which can spill over to adjacent crops (Cahenzli et al., 2018;

Haro-Barchin et al., 2018; Hennig & Mazzi, 2018; Kenis et al., 2016; Klick et al., 2016; Leach et al., 2019; Mazzetto et al., 2020; Pelton et al., 2016; Santoiemma et al., 2019; Tonina et al., 2018; Weissinger, Schrieber, et al., 2019). Considering such surrounding landscape elements, we were able to predict zones of higher infestation risk in nearly all sampled vineyards. Similarly, it is well known that *D. suzukii* avoids staying in dry, hot and sunny places and prefers shady and humid habitats (Diepenbrock & Burrack, 2017; Eben et al., 2018; Enriquez & Colinet, 2017; Evans et al., 2018; Kinjo et al., 2014; Ryan et al., 2016; Tochen et al., 2016; Winkler et al., 2020). It is therefore not surprising that we observed a higher number of eggs laid in the interior of grape clusters than in the outer berries, as the shady interior provides more suitable conditions. These two behavioural findings help to optimize the monitoring of grape infestation. If the goal is to find the first laid eggs in a vineyard or alternatively a high number of eggs, sampling should be concentrated on berries from the interior of grape clusters collected in the zone of highest infestation risk. However, if the aim of the monitoring is to determine the actual infestation of a vineyard, berries should be collected and inspected representatively for the actual plot. Thus, grapes should be collected all over the vineyards and berries should be inspected from all parts of the cluster. Based on the circumstance that about half of the berries are situated in the interior of a cluster (O. Vonlanthen unpublished data), we decided to develop the herein described novel method. Briefly, we decided to inspect the same number of berries from the interior and exterior of grape clusters. We also decided to cut and collect entire grape clusters in order to obtain berries from the interior without damaging a large number of clusters. However, to keep the economic losses for the winegrowers within limits, we opted for collecting only five grape clusters per vineyard at a time and for sampling them in a representative manner for the plot. Thereafter, we selected at random five intact berries from the interior and the exterior of each cluster resulting in totally 50 inspected berries per sample. We named this novel method the 'whole cluster method'.

## 3.2 | Comparison of egg sampling methods

In 2018 and 2019, the three sampling methods 'single berry', 'cluster fragment' and 'whole cluster' were compared with each other in 35 different plots at a total of 87 sampling occasions always inspecting 50 intact berries per sample (Table 1). The estimated loss of yield for the winegrower in a plot was the lowest with the 'single berry method' and around 5 and 11 times higher with the 'cluster fragment' and the 'whole cluster method', respectively. The amount of work for the three methods was about the same since the time gained with the 'whole cluster method' to collect grapes in an actual vineyard was counterbalanced by a longer preparation time to obtain 50 representative berries for inspection in the laboratory (Table 1). Thus, the total amount of time to collect and inspect berries was estimated as 18, 19 and 20 min for the 'whole cluster', 'cluster fragment' and 'single berry method', respectively. Grape berries with eggs of *D. suzukii* were collected with the 'whole cluster method' in 34 of the 87 samples, while only at 20 and 26 sampling occasions with the 'single berry' and 'cluster fragment method', respectively. With 6 against 0 cases, the 'whole cluster method' found in significantly more plots the first egg depositions compared with the 'single berry method', and with 3 cases the 'cluster fragment method' situated in between them (Table 1). Similarly, the 'whole cluster method' identified as the only method any infestation at 8 sampling occasions, whereas the same was true in only 1 and 3 cases for the 'single berry' and 'cluster fragment method', respectively. With 22 against 3 cases, the 'whole cluster method' monitored at significantly more sampling occasions the highest rate of infestation compared with the 'single berry method', while the 'cluster fragment method' situated in between them (Table 1). Moreover, the average percentage of infested berries was significantly higher with the 'whole cluster method' than the other two methods. Thus, the sensitivity for the calculated mean infestation of the 'whole cluster method' was 1.3 times and 1.6 times higher than for the 'cluster fragment' and the 'single berry method', respectively (Table 1).

**TABLE 1** Summary statistics for the three sampling methods. The weight of a sample as well as sampling time in the field and inspection time in the laboratory were measured on a single occasion. The last column presents the *P*-value and the test statistics. Values with different letters were significantly different in Bonferroni pairwise comparisons ($p < .05$)

| | **"Single berry method"** | **"Cluster fragment method"** | **"Whole cluster method"** | ***p* (test statistics)** |
|---|---|---|---|---|
| *N* samples taken in 2018 and 2019 | 87 | 87 | 87 | |
| *N* berries checked/sample | 50 | 50 | 50 | |
| Approximate weight of a sample (g) | 112 | 600 | 1200 | |
| Approximate sampling time in the field (min) | 10 | 7 | 4 | |
| Approximate inspection time in the laboratory (min) | 10 | 12 | 14 | |
| *N* samples with egg detection | 20*a* | 26*a* | 34*a* | 0.16 ($\chi^2 = 3.7$) |
| *N* samples with first egg detection for a plot | 0*a* | 3*ab* | 6*b* | 0.05 ($\chi^2 = 6.0$) |
| *N* samples without egg detection by other two methods | 1*a* | 3*a* | 8*a* | 0.04 ($\chi^2 = 6.5$) |
| *N* samples with highest infestation | 3*a* | 9*ab* | 22*b* | <0.001 ($\chi^2 = 16.6$) |
| Measured range of infestation in % | 0 to 50 | 0 to 40 | 0 to 68 | |
| Mean infestation in % (±SE) | 5.9*a* (±2.2) | 7.3*a* (±2.3) | 9.6*b* (±2.9) | <0.001 ($F_{2,50} = 9.4$) |
| Sensitivity compared with the "Whole cluster method" | 0.62 | 0.76 | 1 | |

The 'whole cluster method' was the most sensitive sampling method since its calculated mean infestation rate was highest and it identified eggs earlier and in more samples than the two traditional and widely used 'single berry method' (Kehrli, Cahenzli, et al., 2017; Mazzetto et al., 2020) and 'cluster fragment method' (Weissinger, Schrieber, et al., 2019). This higher sensitivity originates from the fact that a higher number of berries from the interior of grape clusters is inspected than with the other two methods. As stated earlier, *D. suzukii* avoids to stay in dry and sunny places and females prefer to lay eggs in the shady cluster interior (Diepenbrock & Burrack, 2017; Eben et al., 2018; Tochen et al., 2016). Although statistically not significant, this finding is also supported by a field observation of Van Timmeren and Isaacs (2014) who reared thrice the number of *D. suzukii* adults per gram of grapes from cluster samples compared with individual berry samples. Considering workload, there are no relevant differences between the three methods. The only drawback of the novel 'whole cluster method' is that crop loss is higher than with the two traditional methods, since it is unfortunately not feasible to obtain berries from the interior of clusters without injuring surrounding berries and consequently making them more vulnerable to the development of diseases such as grey mould and sour rot. Nonetheless, we believe that this inconvenience is largely compensated by its higher sensitivity as winegrowers can easily tolerate yield loss equivalent to about one bottle of wine per sampling occasion. The gained information on the health status of a whole vineyard and on the potential need to take additional control measures (e.g. the application of repellent stone powders (Linder et al., 2020) or conventional insecticides (Kehrli et al., 2017; Knapp et al., 2019; Linder & Kehrli, 2019)) is certainly worth this economical loss to a more effective estimation of infestation.

Since 2019, the 'whole cluster method' is used in the Swiss national monitoring programme for *D. suzukii* as the standard method in vineyards. So far, we only had positive feedback and practitioners like, in particular, the rapid and easy manner to collect grape samples in their vineyards, which they frequently send to local advisers for further inspection. Moreover, these advisors noted that they now receive grape samples in better condition for inspection since the transport and storage of five grape clusters is less fragile than that of individual berries or cluster parts. We therefore believe that our novel 'whole cluster method' is currently the easiest and most effective method to assess *D. suzukii* infestation in commercial vineyards.

## 4 | CONCLUSIONS

Female flies lay the highest number of eggs within a vineyard on berries in the interior of grape clusters close to *D. suzukii* favourable landscape elements such as hedgerows, forests, orchards and gardens. This finding might not only be helpful to find the first eggs laid in a vineyard, but might also be used to plan and evaluate efficacy trials in the future. However, when the aim is to monitor and determine the actual infestation of a vineyard, berries should be collected and inspected in a representative manner for the actual plot. The more sensitive 'whole cluster method' meets this demand and is therefore certainly a valuable asset in the monitoring and management of *D. suzukii* in vineyards. The observation of egg depositions in grape berries is the only reliable information to estimate the pest's actual infestation pressure at an early stage (Weissinger, Schrieber, et al., 2019) and consequently a valuable asset and base for Integrated Pest Management (IPM). However, this is only a first step in the implementation of efficient and sustainable IPM strategies against this pest. Indeed, since the actual damage is not directly caused by *D. suzukii* but rather by secondary infections with sour rot, the next questions to be addressed are under which meteorological conditions does *D. suzukii* infestation trigger the development of this disease in grapes and when are control measures cost-effective. In fact, most eggs dry shortly after their deposition and only few larvae hatch. Thus, only a small proportion of the laid eggs actually trigger the development of sour rot (Ioriatti et al., 2018; Rombaut et al., 2017). Moreover, sour rot relies on very specific weather conditions to develop (Huber, 2016; Viret, 2014), conditions that are only occasionally met (Entling & Hoffmann, 2020). Finally, the cost-effectiveness of an intervention depends on the efficacy of the control measure, the duration until harvest and the marketable value of the vintage. Pesticide applications against *D. suzukii* and sour rot are commonly of limited efficacy in vineyards (Hall et al., 2018; Kehrli, Cruchon, et al., 2017; Knapp et al., 2019; Linder et al., 2020). When grapes are nearly mature, it is therefore frequently recommended to advance the date of harvest. At last, the loss of income that a certain level of sour rot infestation may cause is directly dependent on the achieved revenue of the final product. Now that monitoring of the pest has been improved, these aspects need further clarification in order to develop sustainable and cost-effective IPM strategies against *D. suzukii* in vineyards.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHOR CONTRIBUTION

PK, JM, OV, CC and CL designed the study. PK, JM, OV, CC, LJ, TS and NS conducted the surveys. PK and JM analysed the data, and PK wrote the initial manuscript. PK, JM, OV, CC, LJ, TS, NS and CL revised and approved the manuscript.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available at: https://doi.org/10.5281/zenodo.5181421

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